

INTRODUCTION TO GLOMERULAR DISEASES

Goal: to explain the general mechanisms leading to glomerular diseases and to analyze what is known about their relationship to morphologic and clinical manifestations of glomerular injury

Learning objectives

On completion of this lecture, the listener should be able to:

1. explain the pathogenesis of glomerular injury related to the formation of antigen-antibody complexes *in circulation* and *in-situ*
2. explain the pathogenesis of glomerular disease in injury by antibody directed against tissue antigens
3. explain the impact of injury to epithelial cells on glomerular filtration
4. contrast and compare the different pathomechanisms of glomerular injury and explain how they correlate with different patterns of glomerular injury and different clinical syndromes
5. identify the role of genetics in glomerular diseases
6. explain the terms: glomerulonephritis, glomerulopathy, nephropathy, sclerosis ...

Additional resources:

- Robbins Basic Pathology, 9th edition, chapter 13, pages 517-523
- Review: Robbins Basic Pathology, 9th edition, chapter 2, pages 50-52, chapter 4, pages 109-110, 114-117
- Review – 1st year “Host Defense” course
- recorded urinary tract histology, part I (independent study)

OUTLINE

1. Kidney diseases in general and glomerular diseases – the context
2. Glomerular histology – brief review
3. Pathogenesis of glomerular diseases: immune complex versus other
4. Pathophysiology of immune complexes (refresher) and concept of proximal versus distal zones
5. Circulating and in-situ immune complex mediated disease: postinfectious glomerulonephritis and membranous nephropathy
6. Anti- glomerular basement membrane-mediated glomerulonephritis, crescent
7. Other mechanisms: podocyte injury – minimal change and focal and segmental glomerular sclerosis
8. Genetics and glomerular diseases: Alport syndrome and congenital nephrotic syndrome
9. Summary
10. vocabulary

Kidney diseases – bird's eye view:

Each year in the US >100,000 people are diagnosed with end stage renal disease
CDC estimates that >10% of **adults** in the US (>20 million people) may have chronic kidney disease, of varying levels and seriousness

Causes of kidney failure: - prerenal, - intrarenal, - postrenal

Glomerular diseases fit into a much bigger category of intra renal diseases and are actually much less common than diabetes and hypertension. However, their evaluation via biopsy plays a major role in the selection of therapies.

Histology review

Glomerular tuft = network of capillaries: lined by endothelium, held together by mesangium (consisting of cells & matrix) and surrounded by the basement membrane. The latter separates the endocapillary space from the extracapillary space. In the latter there are 2 types of epithelium: visceral (aka “podocytes”) and parietal epithelial cells. The glomerular cells can react to injury in different ways including phagocytosis (mesangial cells), proliferation (mesangial, endothelial, parietal epithelial) or loss (podocytes). All of these processes may impact on blood/primary filtrate flow
Injury to glomerular basement membrane may lead to the loss of structural integrity (with hematuria) or loss of selective filtering (with proteinuria) or, at times, both.

Pathogenesis of glomerular diseases

Many diseases are immune complex mediated and can be caused by circulating or in-situ immune complex formation or by antibody against tissue antigen. To study these mechanisms we need immuno stains and electron microscopy

Other mechanisms are less well studied/known and include soluble mediators and cells
The role of genetics in pathogenesis of glomerular diseases is increasingly recognized and may include abnormalities in immunologic response or structural abnormalities.

Pathophysiology of immune complexes and concept of proximal versus distal zones

Formation of immune complexes does NOT ALWAYS lead to disease; rather, the outcome of immune complex formation depends on the following:

- Size of the immune complexes (small or intermediate size = most pathogenic)
- Intensity and the duration of antigen exposure: acute (one shot) versus chronic
- Functional status of the mononuclear phagocytic system
- Other: charge of the complex, valency (antigen), avidity (antibody), affinity of the antigen to tissues, immune complex three-dimensional structure, hemodynamic...
- Increase in vascular permeability, immune complex binding to inflammatory cells (Fc or C3b receptor)
- Dynamic interplay between the antigen and the host response

Pathology of circulating immune complexes

Pathogenesis of systemic immune complex-mediated disease (type III hypersensitivity disease) – 3 sequential phases in disease development Fig. 4-11, p.116

Phase I: formation of antigen-antibody complexes

Phase II: immune complex deposition, complement activation and leukocyte recruitment

Phase III: inflammatory reaction & tissue injury at the site of deposition, *vasculitis, glomerulonephritis, arthritis “innocent bystander” (or by “friendly fire”) tissue injury*
Antibody has no specificity to glomerular components !!!

Factors influencing glomerular localization of antigen, antibody and immune complexes include their molecular charge and size.

Glomerular filtration barrier has 2 layers with negative charge. Highly cationic complexes become sub-epithelial while highly anionic complexes become sub-endothelial and those with neutral charge deposit in the mesangial matrix. Large complexes are cleared by mononuclear phagocyte system and usually are not nephritogenic. However, if not cleared, they become sub-endothelial. Deposition of these proteins is also influenced by glomerular hemodynamics, mesangial function and the integrity of charge-selective barrier.

Concept of the filtration barrier and distinction between the proximal and distal zones:

- complexes deposited in the proximal zones of the glomerular basement membrane (endothelium, sub-endothelium) elicit an inflammatory reaction in the glomerulus with infiltration of leukocytes and structural injury of the filtration barrier with hematuria
Simplistic explanation: these complexes are “seen” by the circulating inflammatory cells and hence elicit their reaction with injury and engagement of the endocapillary cells - mesangial and endothelial.

- antibodies directed to distal zones of the glomerular basement membrane (epithelium and subepithelium) form complexes in the distal zone and are largely non-inflammatory but affect podocytes (visceral epithelial cells) with alteration of the filtration barrier and consequently cause proteinuria. The complexing of the antigen and antibody in the subepithelial space is unique, because the binding occurs on the urinary side of the glomerular basement membrane. The site of the deposit is remote from the activators that are normally present in the circulation.

Simplistic explanation: these complexes are “not seen” by the circulating cells, hence there is no inflammatory reaction, no endothelial injury or mesangial engagement.

Dynamics of immune complex formation:

- in antibody excess large immune complexes are formed which are rapidly phagocytized
- in slight antigen excess small/intermediate immune complexes are formed which bind less avidly to phagocytic cells and circulate longer

- in mononuclear phagocytic system overload or intrinsic dysfunction the immune complexes persist in circulation and there is a greater likelihood of their tissue deposition

Circulating immune complex glomerulonephritis: postinfectious glomerulonephritis

- caused by trapping of circulating antigen-antibody complexes within glomeruli because of physio-chemical properties & hemodynamic factors peculiar to the glomerulus. Antibodies have **NO** immunological specificity for glomerular constituents
- antigens can be either exogenous (infectious) or endogenous (in autoimmune diseases such as Systemic Lupus Erythematosus)
- antigen-antibody complexes cause tissue damage by eliciting an inflammatory reaction at the site of deposition

This is a type III hypersensitivity reaction; experimental model = serum sickness.

Tissue injury at the site of immune complex deposition, i.e. the glomerular capillary wall:

- inflammatory reaction with influx of polymorphonuclear leukocytes (PMNs) leads to proliferation of the mesangial and endothelial cells, leading to progressive narrowing of glomerular capillaries with diminished blood circulation (clinically accumulation of metabolic products with elevation of serum creatinine, i.e. renal failure; retention of water with edema and hypertension), and also
- **STRUCTURAL** damage to the integrity of the glomerular capillary wall with “Swiss cheese” holes. The latter lead mainly to hematuria with only some proteinuria.

Clinically, this process is manifested in the **NEPHRITIC SYNDROME**: hematuria, mild proteinuria, edema, renal failure, hypertension (HTN).

Pathology: influx of PMNs, endocapillary proliferation, immune complexes are detectable by immunofluorescence and electron microscopy as small granules. See also lecture II.

What is the goal of the inflammatory response?

Degradation of immune complexes by neutrophils, monocytes/macrophages and mesangial cells leads to healing phase with complete resolution in most patients.

In-situ immune complex deposition with antigens on basal surface of epithelial cells: membranous nephritis

Reaction of antigen with an antibody on basal surface of epithelial cells causes injury to epithelial cells with foot process effacement and severe proteinuria.

Since the antibodies are directed to distal zones of the glomerular basement membrane (epithelium and subepithelium) this process is largely non-inflammatory and consequently there is **NO** cellular reaction.

As discussed above, the complexing of the antigen and antibody in the subepithelial space is unique, because the binding occurs on the urinary side of the glomerular

basement membrane. The subsequent activation of complement and cytokine factors is modified, because the site of the deposit is remote from the activators that are normally present in the circulation. The reduced response produces a lesion that looks “benign” on light microscopy (i.e. no inflammatory cells, no endocapillary proliferation). The terminal complement membrane attack complex (C5b-9) is detected in urine because complement deposition and activation occurs in the urinary space after the immune complex has been formed.

What is the clinical picture?

- No inflammatory response – “no Swiss cheese” or “sieve” injury”
 - subepithelial immune complex deposition impacts the filtration integrity of the epithelial cells and glomerular basement membrane
- “gauze-like or fine mesh effect”: there is NO permeability to red blood cells but heavy proteinuria
- CLINICALLY = NEPHROTIC SYNDROME
- Nephrotic syndrome = heavy proteinuria + s/s of protein depletion
- Human disease: membranous nephropathy

Membranous nephropathy can be reproduced experimentally in a model known as Heymann nephritis

- In this model rats are injected with antigen consisting of proximal tubular brush border. Animals develop antibodies against the brush border antigen. These antibodies also react with the basal surface of epithelial cells. Complement activation ensues and shedding of the immune complexes from cell surface to sub-epithelial location occurs and granular sub-epithelial deposits are detectable by immunofluorescence (and electron microscopy). In humans this nephropathy is an autoimmune process. See also lecture III.
- This type of antibody reaction can also occur with antigens planted along the epithelial aspect of the glomerular basement membrane such as viral, bacterial, drugs...

While this experimental model of membranous nephritis was known since 1959, its validation was achieved only recently (*NEJM*, 2002, 346:2053). This was possible following the discovery of several families with neonatal nephrotic syndrome and membranous nephropathy. Subsequent evaluation showed that mothers had mutations in neutral endopeptidase (NEP, normal podocyte antigen). With pregnancy, the mother has formed antibodies to NEP antigen of the fetus. These anti-NEP antibodies crossed the placental barrier to the fetus and led to the in-situ formation of immune complexes in the sub-epithelial space of the glomeruli in the kidney of the fetus, with resultant damage to the glomerular basement membrane and the clinical findings of nephrotic syndrome in the newborn. These observations validated the in situ paradigm in human membranous nephropathy. Proteomic analysis of the target antigen in human “idiopathic” membranous nephropathy showed it to be the phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain containing 7A (THSD7A). It has been shown that most of the reactivity to PLA2R resides in the IgG4 subclass

However, while our understanding of membranous nephropathy continues to evolve, it is apparent that this nephropathy does not fit well into the classification scheme of hypersensitivity diseases (i.e. it is neither a good example of type III nor of type II).

Moreover, the different pathways of glomerular injury (by circulating or in-situ immune complex formation) are not mutually exclusive and in humans more than one mechanism may contribute to injury. For example, in the case of postinfectious glomerulonephritis, in addition to glomerular injury caused by circulating immune complexes, there is also a component of in-situ immune complex deposition. The latter leads to the formation of large subepithelial deposits, “humps” which are unique to postinfectious glomerulonephritis and therefore are diagnostically very useful.

Antibody directed against tissue antigens: anti-glomerular basement membrane glomerulonephritis

Anti-glomerular basement membrane antibody mediated glomerulonephritis is an example of the type II hypersensitivity reaction, where the antigens are present on the cell surface or within the matrix (glomerular basement membrane), and there is direct injury to cells/matrix by an antibody-mediated process.

The deposition of antibodies in fixed tissues (such as glomerular basement membrane) activates complement, generates by-products (including chemotactic agents) that attract leukocytes (PMNs) and monocytes, which, together with anaphylatoxins C3a and C5a, increase vascular permeability. Leukocytes release variety of pro-inflammatory substances, lysosomal enzymes, including proteases capable of digesting basement membrane (see Robbins, page 115, fig. 4).

Antibodies with specificity for glomerular basement membrane bind diffusely along its length as evidenced by linear stain for IgG seen by immunofluorescence. Because the antigens are very fine and present throughout the entire glomerular basement membrane, the actual complexes cannot be seen by electron microscopy. However, their deposition causes severe damage to the glomerular basement membrane with extensive injury along its entire length similar to the “sieve effect”. Since “holes” in this sieve-like injured basement membrane are big enough to let a large number of the red blood cells through, there is usually GROSS hematuria.

CLINICALLY: nephritic syndrome + RAPID renal failure aka:

RPGN, i.e. **R**apidly **P**rogressive **G**lomerulo**N**ephritis

Glomerular crescent = proliferation of the parietal epithelial cells, forming crescent-shaped structure which obliterates entry to the proximal tubule, and which is seen in conjunction with severe destruction of the glomerular capillary wall with severe bleeding. It prevents bleeding into lower urinary tract and hence acts like a “glomerular stopper”. The thus-achieved control of blood loss comes at a price: crescents stop blood loss but also compress the tuft, reduce filtration and lead to rapidly progressing renal failure.

Experimental model, known as “Masugi nephritis” provides an insight into what happens in humans. In this model, rats are injected with anti-rat kidney antibodies (prepared in rabbits) with linear deposition of the antibody (IgG) along the glomerular basement membrane. In humans, the antigen involved in this process has been identified as a noncollagenous domain (NC1) of the $\alpha 3$ chain of collagen type IV which under normal circumstances is encrypted and does not elicit an autoimmune response. See also lecture II. Interestingly, these antibodies also cross-react with alveolar basement membrane in the lung causing pulmonary hemorrhage and the combination of this type of kidney and lung injury known as Goodpasture syndrome.

Immune mechanisms causing glomerular injury - summary comments:

Immune complexes can form in circulation or in-situ.

In glomerulonephritis caused by circulating immune complexes, antibodies have NO immunological specificity for glomerular constituents

In in-situ immune complex deposition, antibodies have immunological specificity for glomerular constituents, which can be either fixed intrinsic tissue antigens (autoimmunity) or planted antigens (exogenous or endogenous).

In the case of antibody directed against tissue antigens, anti-glomerular basement membrane antibody binds *diffusely* along the glomerular basement membrane (linear stain for IgG [antibody]) with severe damage to the glomerular basement membrane, with GROSS hematuria; there is the formation of crescents and a clinical picture of rapidly progressive glomerulonephritis.

The type of glomerular response to injury and the clinical picture are largely dependent on the localization of the immune complexes in the glomeruli:

- endothelial/sub-endothelial antibodies/complexes – inflammatory reaction
- epithelial/sub-epithelial antibodies/complexes – non-inflammatory

Inflammatory injury is clinically associated with hematuria and NEPHRITIC syndrome

Non-inflammatory injury is clinically associated with proteinuria and NEPHROTIC syndrome. However, other mechanisms (non-immune complex mediated) of glomerular injury can also lead to proteinuria and nephrotic syndrome as discussed below.

Other mechanisms of glomerular injury – NOT caused by detectable antibodies – podocytes injury: minimal change disease, focal and segmental glomerular sclerosis (FSGS)

Other mechanisms of glomerular injury where we do NOT detect antibodies by standard techniques (immunofluorescence, electron microscopy) and which are **less well known** include the following mediators:

- cells: neutrophils, monocytes, macrophages, resident glomerular cells
T lymphocytes, NK cells, platelets,
- soluble mediators: complement, eicosanoids, NO (nitric oxide), angiotensin, endothelin, cytokines, chemokines (PDGF [Platelet-derived growth factor] induces proliferation, TGF- β [Transforming growth factor-beta] induces the synthesis of various extracellular matrix proteins), coagulation system

HEAVY PROTEINURIA/NEPHROTIC SYNDROME can develop following isolated injury to visceral epithelial cells (podocytes) by:

- putative antibodies to podocytes: cytokines ? or a not-yet-characterized “permeability factor”, which may cause recurrence of proteinuria post kidney transplantation
- injury to podocytes may be reversible or seemingly irreversible. In this context, one should remember that mature podocytes do NOT proliferate; hence, irreversible podocyte injury leads to scarring

Among the various experimental models of epithelial cell injury are those involving toxins (puromycin) or hyperfiltration (partial nephrectomy)

Pathology of podocyte injury:

- reversible, manifested by effacement of the epithelial cell foot processes (minimal change disease)
- irreversible, associated with podocyte detachment, death and progressive obliteration of the affected capillary termed “sclerosis”(focal and segmental glomerular sclerosis).
- alteration of the glomerular basement membrane charge with loss of its negative charge and consequently permeability to negatively charged proteins such as albumin with severe proteinuria.

Podocyte injury (podocytopathies) lead to heavy proteinuria which is a hallmark of the NEPHROTIC syndrome. You may recall that the nephrotic syndrome can also be caused by subepithelial immune complex deposition.

Genetic defects:

- (i) affecting immune response
- (ii) structure

Mutations in genes encoding:

- type IV collagen with hematuria (Alport syndrome)
- slit diaphragm proteins with nephrotic syndrome: *NPHS1* encoding *nephrin* – congenital nephrotic syndrome of the Finnish type, *NPHS2* encoding *podocin* – steroid-resistant nephrotic syndrome of childhood onset

Pathogenesis - summary:

(i) NEPHRITIC SYNDROME - **HEMATURIA**

- circulating immune complexes – **postinfectious glomerulonephritis** (serum sickness model)
- anti-glomerular basement membrane antibody mediated GROSS hematuria/rapidly progressing renal failure – **anti-glomerular basement membrane disease** (nephrotoxic serum [Masugi] nephritis)

(ii) NEPHROTIC SYNDROME – **PROTEINURIA**

- in-situ immune complex formation – **membranous glomerulonephritis** (Heymann nephritis model)
- podocyte injury non-immune complex mediated
reversible = **minimal change disease**
irreversible = **focal and segmental glomerular sclerosis (FSGS)**

Genetics: defects affecting (i) immune response, (ii) structure

Different pathways of glomerular injury are not mutually exclusive and in humans more than one mechanism may contribute to injury.

Host factors, which usually are also not static, are also critical to determining who does, and who does not, develop glomerular disease.

Thus, the disease is a dynamic process, more akin to a movie rather than a snap-shot

During the subsequent lectures, I will discuss the specific clinical entities associated with nephritic syndrome (lectures I and II) and nephrotic syndrome (lectures II and III).

VOCABULARY

Glomerular diseases usually have the “**glomerulo**” prefix: – see postinfectious glomerulonephritis etc

Glomerulonephritis is used preferentially in reference to glomerular diseases with an inflammatory/proliferative response

Glomerular pathologies, without an inflammatory response may be referred to as “nephropathy” or “glomerulopathy” - see membranous nephropathy (glomerulopathy)

Nephrosis is meant to indicate a non-inflammatory nephropathy, which is associated with nephrotic syndrome

“nephritis” can also be attached/used in connection with other kidney diseases, such as “pyelonephritis”

Greek: nephros = kidney, nephrologist = MD specializing in medical kidney diseases
Urologist takes care of “surgical ” kidney diseases such as tumors, reflux, etc.

Sclerosis:

Glomerular sclerosis: increased collagenous extracellular matrix that is expanding the mesangium, and subsequently obliterating the capillary lumen, or forming adhesions with the Bowman’s capsule

Vascular sclerosis:

Hyaline arteriosclerosis: hyaline (proteinaceous) deposits with thickening of the wall and narrowing of the lumen of small arteries, i.e. “arterioles”

Hyaline from Greek: *crystal, glass*. A hyaline substance appears glassy and pink in H&E stain

Arteriosclerosis: “hardening of the arteries”, wall thickening and loss of elasticity

Nephrosclerosis: “hardening” of the kidney due to vascular disease

H&E stain (hematoxylin & eosin stain) = routine pathology stain in which cytoplasm is pink (owing to staining with eosin) and nuclei dark blue (owing to staining with hematoxylin)